10X GENOMICS SINGLE CELL GENE EXPRESSION
Sample Submission Guidelines
Chromium Single Cell Multiome & ATAC

The quality of the nuclei suspension to be used with the Multiome and ATAC methods from 10X Genomics plays a crucial role in obtaining high quality sequencing data of captured nuclei. Please visit 10X Genomics webpage for "Demonstrated Protocols" on how to obtain high quality single nuclei suspensions based on your starting material (https://www.10xgenomics.com/support/single-cell-multiome-atac-plus-gene-expression) for Multiome run and (https://www.10xgenomics.com/support/user-guides/single-cell-atac?menu[productNames][0]=Single%20Cell%20ATAC) for ATAC run.

ESCG recommends that you test the protocol to generate your nuclei suspension before scheduling an actual run with the 10X methods. It is important that you show ESCG the quality of the suspension by either sending a representative picture of the whole suspension (several nuclei in the image field) by email, or that you book a time to bring the suspension to the facility for evaluation.

In order to obtain high quality data from your experiment the nuclei suspension should have:

- Been prepared in low-binding tubes
- A viability of the cell suspension of more than 90% (before nuclei isolation)
- High-quality nuclei have well-resolved edges
- No aggregates
- No observable debris
- No inhibitors of reverse transcription or GEM generation
- Enough extra nuclei suspension for counting them, to perform accurate nuclei loading
- Smallest possible volume for suspensions coming from FACS

Storage:
Freezing or cryopreservation of nuclei is not recommended as freezing can damage cellular/nuclear membranes. Furthermore, since nuclei are permeabilized, freezing can increase the potential of nuclear leakage and increase the background signal. Cryopreservation at the cell or tissue stage is recommended as an alternative to the freezing of nuclei.

Important

- Please check the quality of your samples, be sure that there are no aggregates and debris.

- Please count your samples with an accurate automated cell counter or manually before submission to the facility. Nuclei being smaller in size may be more difficult to count. This can be further exacerbated if there is lots of cellular debris present, as common counting dyes like Trypan Blue may also stain debris. The use of a nucleic acid staining fluorescent dye (like AO/PI or Ethidium Homodimer-1) and a fluorescent capable automated counter or microscope may help improve nuclei counting accuracy as only nucleic acids will be stained and not debris. It is best to count in replicates to ensure accuracy. Please see 10X Genomics' Technical Note with Guidelines on Accurate Target Cell Count

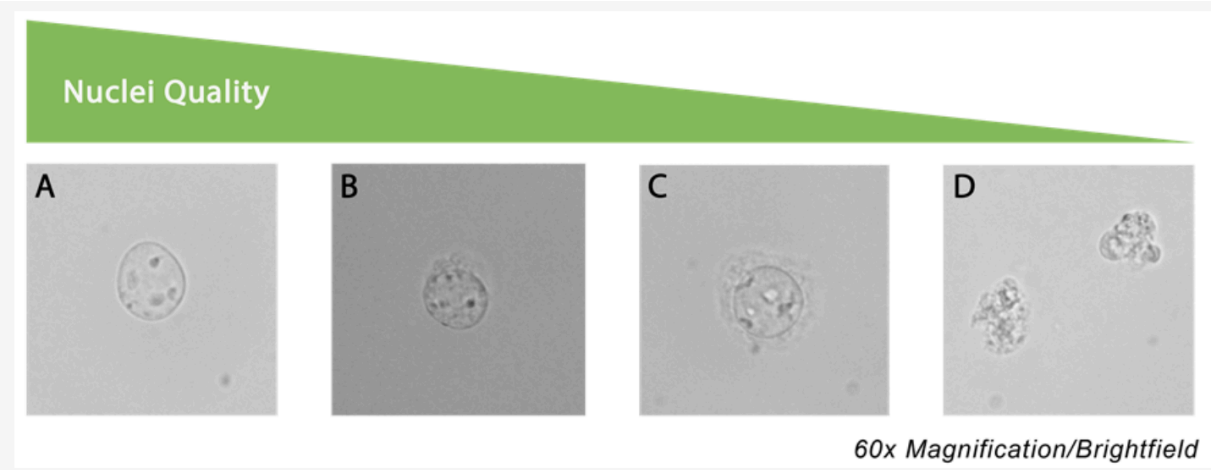
- Before the run, you need to collect nuclei buffer from the ESCG facility. The concentration of the buffer is 20X and it needs to be diluted by 1:20 in nuclease-free water before use.

- If the user insists on going forward with the protocol even though they have suboptimal cell or nuclei suspensions, the user will be required to sign a form confirming that they have received information about low sample quality and will be invoiced regardless of the resulting library and data quality. The person who brings the sample must have permission to make the decision of proceeding with the samples, or if they need, contact the responsible person to get confirmation.

Explanations to High Quality Nuclei Suspension Requirements

Nuclei:
Ideally, the nuclei suspension should contain **more than 90% intact nuclei**. After nuclei isolation, the nuclear membrane should also be visualized under a microscope at 40x or 60x magnification to ensure no blebbing. High-quality nuclei have well-resolved edges. To minimize physical damage to nuclei from shearing forces, it is critical to pipette gently and slowly during nuclei resuspensions. Nuclei suspensions should be loaded on the Chromium chip as soon as possible after preparation, ideally **within 30 min**.

Examples of nuclei quality below. More information is found at:
https://kb.10xgenomics.com/hc/en-us/articles/360050780051-What-are-the-best-practices-for-working-with-nuclei-samples-for-3-single-cell-gene-expression-



A: High-quality nuclei have well-resolved edges. Optimal quality for single-cell gene expression libraries.

B: Mostly intact nuclei with minor evidence of blebbing. Quality single-cell gene expression libraries can still be produced.

C: Nuclei with strong evidence of blebbing. ***Proceed at your own risk.***

D: Nuclei are no longer intact. ***Do not proceed!***

For protocols to isolate nuclei for Single Cell Multiome ATAC and Gene Expression, refer to the following demonstrated protocols:
https://support.10xgenomics.com/single-cell-multiome-atac-gex/sample-prep

For the Multiome assay, 10X Genomics recommends a concentration of 1U/ul of RNAse inhibitor in all buffers. 10X Genomics strongly recommends Protector RNase Inhibitor from Sigma Aldrich (PN-3335399001).
In case of no availability for Protector RNase Inhibitor from Sigma Aldrich (PN-3335399001), refer to the following 10x Genomics information:
https://kb.10xgenomics.com/hc/en-us/articles/360049543672-Can-I-use-an-alternative-RNase-inhibitor-part-number

Nuclei aggregates and debris:
Nuclei aggregates can cause clogging of the 10X microfluidic chip and will increase the likelihood of having doublets/multiples of nuclei. Debris in your nuclei suspension can cause clogging of the 10X microfluidic chip, contamination of your single nuclei with ambient RNA, and inaccurate nuclei counting. If you see debris in your nuclei suspension, please use a strainer.

Nuclei clumping could be a sign of overlysis. This can be avoided by using optimal lysis conditions (see above). Nuclei suspensions can also be filtered to reduce clumps. See: What cell strainers are recommended?

Sorting of nuclei to remove debris can be performed, however, extra care should be taken to avoid nuclei damage as additional handling of nuclei can be stressful and

further compromise the nuclear membranes, leading to leakage of nuclear content. Additionally, certain sorting dyes can alter chromatin structure: Can I sort nuclei for Single Cell ATAC sequencing? Follow recommended sorting guidelines as described in the nuclei isolation demonstrated protocols.

Multiplets

The multiplet rate is the fraction of nuclei-associated barcodes estimated to be associated with more than one nucleus. Please keep in mind that multiplets cannot always be removed bioinformatically.

Multiplet Rate

Multiplet Rate (%)	# of Nuclei Loaded	# of Nuclei Recovered
0.4%	~775	~500
0.8%	~1,550	~1,000
1.6%	~3,075	~2,000
2.3%	~4,625	~3,000
3.1%	~6,150	~4,000
3.9%	~7,700	~5,000
4.6%	~9,250	~6,000
5.4%	~10,750	~7,000
6.2%	~12,300	~8,000
6.9%	~13,850	~9,000
7.7%	~15,400	~10,000

10X generally recommends a maximum of 10.000 nuclei/sample. We recommend aiming for a maximum of 7.500 nuclei to decrease the risk of overloading the sample. Exceeding 10.000 nuclei would lead to increased multiplet rates and loss of single cell status of the data. One can expect around +/- 50% load, even more if the sample quality is low.

See below to prepare diluted nuclei buffer for Multiome protocol:

Prepare	Diluted Nuclei Buffer	Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
		20X Nuclei Buffer (PN-2000207)	20X	1X	50 µl
		DTT	1,000 mM	1 mM	1 µl
		RNase Inhibitor (confirm vendor-specific stock concentration)	40 U/µl	1 U/µl	25 µl
		Nuclease-free Water	-	-	924 µl

See below to prepare diluted nuclei buffer for ATAC protocol:

Prepare	Diluted Nuclei Buffer	Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
		20X Nuclei Buffer (PN-2000207)	20X	1X	50 µl
		Nuclease-free Water	-	-	950 µl

Based on the targeted nuclei recovery, resuspend the nuclei in diluted nuclei buffer to get the corresponding nuclei stock concentration. See below table. This enables pipetting volumes of the nuclei stock for transposition to be between 2-5 µl. Higher nuclei stock concentrations will result in lower pipetting volumes that may increase nuclei variability.

Multiome protocol – nuclei stock concentrations

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
500	160-400
1,000	320-810
2,000	650-1,610
3,000	970-2,420
4,000	1,290-3,230
5,000	1,610-4,030
6,000	1,940-4,840
7,000	2,260-5,650
8,000	2,580-6,450
9,000	2,900-7,260
10,000	3,230-8,060

ATAC protocol – nuclei stock concentrations

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
500	155-390
1,000	310-780
2,000	610-1,540
3,000	925-2,300
4,000	1,230-3,075
5,000	1,540-3,850
6,000	1,850-4,600
7,000	2,150-5,400
8,000	2,460-6,150
9,000	2,770-6,900
10,000	3,080-7,700

Prior to loading your sample onto the 10X Chromium chip, we inspect it under the microscope. We request that you stay with us during these steps in case we see a suboptimal nuclei suspension and need to discuss it with you before starting the experiment. In the end, ESCG always advises the best possible outcome based on our experience, but it is the user's decision to proceed or not with a non-optimal sample.

Nuclei number and concentration:

If possible, we request you to bring at least roughly **three times the number of nuclei** you aim for as output, meaning if you aim for having 5,000 nuclei sequenced, we need you to bring 15,000 nuclei.
If your nuclei suspension is too diluted when arriving at the facility, you can use our centrifuges for concentrating it, please remember to bring extra buffer.

When submitting your sample to ESCG, the recommended buffer is Nuclei Buffer 20X which you can pick up from the facility and dilute to 1:20 before use. *On the run day, please bring the diluted prepared Nuclei Buffer with you.*

Possible failures during the 10X method run

Clog

When running samples using the 10X method, occasionally a sample clog may occur. This is generally caused by suboptimal sample preparation (suspension containing clumps or debris), or by loading more than the recommended number of nuclei. If clogging occurs during the generation of the emulsions, these will not be formed properly, and your nuclei will not be encapsulated in single droplets and the processing generally cannot proceed. If the sample suspension was sub-optimal and we had discussed the risk of a clog occurring, then the cost of the run is on the user.

If it is a very precious sample and a re-run cannot be attempted (see below), it may be possible to continue with the protocol and generate cDNA and libraries, although at lower levels and coming from a reduced number of cells.

If the quality of the sample suspension was such that no clogging was to be suspected, then we can relate to a run failure, and this will be covered by the 10X Genomics company.

Wetting failure

Another type of failure that can occur during a sample run is a wetting failure. This often has no obvious cause and will therefore be reimbursed by the 10X Genomics company. In such cases, there will be no cost for the user for the failed sample.

In the case of a wetting failure

If there is a wetting failure or a clog on a clean, high-quality sample, the facility will attempt to re-run the sample **IF** there are enough nuclei and **IF** the facility has reagents in stock. However, we cannot guarantee that a sample will be re-run.

Information regarding low-quality starting material at ESCG

We have been informed by the staff at the ESCG facility that the samples listed below were of suboptimal quality. We chose to continue the 10x genomic sample preparation despite being informed that it may fail or result in low-quality data. If so, we accept that we will still be billed for the run of the sample, as the reagents of the kit will be consumed.

Cell/Nuclei samples with low quality

Sample name	ESCG Sample ID

_____ _____ _____

Date Name Signature